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Toxicity of Copper to Cutthroat Trout ('Salmo clarki') under Different Conditions of Alkalinity, pH, and Hardness

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16. ABSTRACT

Median lethal concentration (96-h LC50) values for acute copper toxicity to 3-10 g cutthroat trout (Salmo clarki) have been determined for nine different combinations of alkalinity, hardness, and pH. Equilibrium calculations were performed on the copper LC50 values; seven different soluble species of copper were considered: Cu^{2+}, $CuOH^{+}$, $Cu(OH)_2^{0}$, $Cu_2(OH)_2^{2+}$, $CuHCO_3^{+}$, $CuCO_3^{0}$, and $Cu(CO_3)_2^{2-}$. The acute toxicity of copper was inversely correlated with water hardness and alkalinity. At a given alkalinity, hardness determined the LC50; at a given hardness, alkalinity determined the LC50. At a given alkalinity and hardness, the concentrations of the copper species were determined by the pH of the water. Under the conditions tested, Cu^{2+}, $CuOH^{+}$, $Cu(OH)_2^{0}$, and $Cu_2(OH)_2^{2+}$ were toxic forms of copper to cutthroat trout; $CuHCO_3^{+}$, $CuCO_3^{0}$ and $Cu(CO)_3)_2^{2-}$ were not toxic. Results of 11 96-h copper toxicity bioassays on 1- to 26-g rainbow trout (Salmo gairdneri) under uniform water chemistry conditions are also reported.

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Toxicity of Copper to Cutthroat Trout (*Salmo clarki*) under Different Conditions of Alkalinity, pH, and Hardness

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Journal Article

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■ Median lethal concentration (96-h LC_{50}) values for acute copper toxicity to 3–10-g cutthroat trout (*Salmo clarki*) have been determined for nine different combinations of alkalinity, hardness, and pH. Equilibrium calculations were performed on the copper LC_{50} values; seven different soluble species of copper were considered: Cu^{2+}, $CuOH^+$, $Cu(OH)_2^0$, $Cu_2(OH)_2^{2+}$, $CuHCO_3^+$, $CuCO_3^0$, and $Cu(CO_3)_2^{2-}$. The acute toxicity of copper was inversely correlated with water hardness and alkalinity. At a given alkalinity, hardness determined the LC_{50}; at a given hardness, alkalinity determined the LC_{50}. At a given alkalinity and hardness, the concentrations of the copper species were determined by the pH of the water. Under the conditions tested, Cu^{2+}, $CuOH^+$, $Cu(OH)_2^0$, and $Cu_2(OH)_2^{2+}$ were toxic forms of copper to cutthroat trout; $CuHCO_3^+$, $CuCO_3^0$, and $Cu(CO_3)_2^{2-}$ were not toxic. Results of 11 96-h copper toxicity bioassays on 1- to 26-g rainbow trout (*Salmo gairdneri*) under uniform water chemistry conditions are also reported.

A variety of environmental factors influence the toxicity of copper to fishes (*1, 2*). Among these factors are pH, hardness, alkalinity, and inorganic and organic complexation. The work of several investigators (*3–9*) indicates that organically bound copper is nontoxic and copper toxicity decreases as the copper(II) ion is chelated. Consequently, the toxicity of copper to fishes is attributed to the inorganic forms of copper.

It is now recognized, as noted by Lloyd and Herbert (*10*), that copper is more toxic in soft water than it is in hard water. Alkalinity and hardness are usually directly related. Stiff (*11*) hypothesized that if copper(II) ion were the toxic form of copper and if the copper carbonate complex, noted by Scaife (*12*), were relatively nontoxic, then the difference in toxicity of copper between soft and hard water would be related to the difference in alkalinity, rather than the hardness, present in those waters. In support of this, Andrew (*13*) stated that bicarbonate alkalinity has a major role in limiting copper toxicity in natural waters. Alkalinity is directly related to pH (*14*); because a relationship can be established between alkalinity and the copper(II) ion activity, pH is also related to the copper(II) ion activity.

Although it is generally accepted that the copper(II) ion is toxic to fishes, it is not the only toxic form of copper. Shaw and Brown (*9*) concluded that Cu^{2+} and $CuCO_3^0$ are the toxic forms of copper to rainbow trout (*Salmo gairdneri*). Pagenkopf et al. (*15*) reported that Cu^{2+} is toxic and $CuOH^+$ may be toxic. Andrew (*13*) stated that toxicity is directly related to the ionic activity of the cupric ion and not to its inorganic complexes. However, in a more recent study (on *Daphnia magna*), Andrew et al. (*16*) state that copper toxicity is directly related to activities of Cu^{2+}, $CuOH^+$, and $Cu_2(OH)_2^{2+}$. Howarth and Sprague (*17*), in work on rainbow trout, also concluded that Cu^{2+}, $CuOH^+$, and $Cu_2(OH)_2^{2+}$ are toxic.

Stiff (*18*) applied an analytical scheme to differentiate the chemical states of copper into Cu^{2+}, $CuCO_3^0$, and Cu(organic). Mancy and Allen (*19*) developed a mathematic model to determine the concentration of each species in the copper–carbonate system. Various computer programs (*20, 21*) are available for evaluation of the equilibrium relationships to predict the speciation of metals in aqueous systems.

This paper reports on a series of bioassays on the toxicity of copper to cutthroat trout (*Salmo clarki*) under different conditions of alkalinity, hardness, and pH; no copper toxicity studies have previously been reported for this species.

Experimental

Nine acute copper toxicity flow-through bioassays on cutthroat trout were conducted at the Montana State University Fisheries Bioassay Laboratory, located at the Bozeman (Montana) Fish Cultural Development Center (FCDC), U.S. Fish and Wildlife Service. Test fish, obtained from FCDC, were a west slope strain reported to be free from any possible hybridization. The source of water for the bioassays was a natural groundwater spring located at FCDC. This water had a hardness of ~200 mg/L as $CaCO_3$, alkalinity of ~175 mg/L as $CaCO_3$, and no other appreciable constituents (Table I). To reduce the hardness and/or alkalinity this water was first passed through cation and/or anion exchange resin columns (Culligan Water Conditioning Co., CH-1 and CS-2), and then mixed with untreated spring water to achieve the desired hardness and alkalinity for each test. In tests in which either hardness or alkalinity were to be increased to levels over those of the mixtures, reagent grade calcium chloride or sodium bicarbonate was added with capillary flow meters or fluid metering pumps. Bioassays were conducted in nine different water combinations (Table II) in a 3×3 experimental design with three concentrations (high, medium, and low) each of alkalinity and hardness similar to the classification developed by the U.S. Geological Survey (*22*).

Reagent grade copper(II) chloride in demineralized water acidified with concentrated hydrochloric acid was used as the toxicant. The test solution was delivered by a proportional diluter (dilution factor = 0.75) having the basic design of Mount and Brungs (*23*) at a flow rate of 500 mL every 2–3 min to each of six copolymer plastic tanks: five test and one control. The water volume of each tank was 62 L.

Copper concentrations in test tanks were determined according to the cuprethol method (*24*) using 10-cm cells. Copper was measured on nonfiltered samples; however, a series of copper analyses was conducted on 0.45-μm filtered and nonfiltered samples of the test waters used in the bioassays. From these analyses appropriate factors (Table III) were obtained so that the concentrations of copper measured on nonfiltered samples could be converted to dissolved copper as defined by the 0.45-μm filter.

Total alkalinity was determined as in "Standard Methods for the Examination of Water and Wastewater" (*25*) with methyl purple as the indicator. Total hardness (EDTA), calcium (EDTA), nitrate nitrogen, and ammonia nitrogen were determined according to analytical procedures described in "Standard Methods" (*25*). Nitrite nitrogen was determined according to the U.S. EPA (*26*). Magnesium was calculated from total hardness and calcium hardness (*25*). Dissolved oxygen was measured using a Y.S.I. Model 54 meter, temperature with a calibrated mercury thermometer, and pH with a Beckman Phasar-I digital meter. Colorimetric measure-

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Table I. Chemical Characteristics of the Untreated Dilution Water Used in Bioassays [a]

alkalinity, as $CaCO_3$	176	Ca	53
hardness, as $CaCO_3$	199	Mg	36
pH	8.0	Na	4.0
temp, °C	9.8	K	0.64
		Al	<1
sp equiv conductance, μmho/cm, 25 °C	328	As, μg/L	1.5
total org C	3.3	Cd	<0.005
turbidity, NTU	1.7	Cr	<0.005
NH_3-N	0.00	Cu	0.008
NO_2-N	0.00	Fe	0.03
NO_3-N	0.23	Hg, μg/L	<0.05
Cl^-	0.44	Mn	0.003
F^-	0.4	Ni	<0.005
PO_4^{3-}	<0.1	Pb	<0.01
SO_4^{2-}	10.0	Se, μg/L	0.8
		Zn	0.01

[a] All values in milligrams/liter unless otherwise noted.

ments were made using a Varian Model 635 ultraviolet–visible spectrophotometer. Organic constituents were not detected when measuring nitrate nitrogen at 275 nm.

A chemical profile of the untreated dilution water is presented in Table I. For each 96-h bioassay, each tank was monitored on an average of 3 times for copper, 5 times for pH, alkalinity, and hardness, and 2 times for calcium, dissolved oxygen, temperature, ammonia nitrogen, nitrate nitrogen, and nitrite nitrogen. A summary of test tank water chemistry data is given in Table II.

Sixty fish were used for each test, 10 per tank. Fish were acclimated to the dilution water in the test tanks for 2–3 days before introduction of toxicant. Prior to the tests, fish were fed a commercial salmon ration three times daily, but were not fed during the acclimation or test periods. Fish were weighed collectively at the beginning of each test and were measured individually for total length as mortalities were observed or at the conclusion of each test. Fish mortalities, as evidenced by complete immobilization and lack of respiration, were recorded at 4-h intervals for the first 36 h and at 12-h intervals thereafter.

The median lethal concentration (LC_{50}) values and their 95% confidence intervals were calculated from the experimental data using a computer program developed for the trimmed Spearman–Karber method (*27*); toxicity curves were computer-generated using LC_{50} and time values from each time of mortality observation.

Speciation of the LC_{50} copper values was accomplished using the computer program COMICS (*20*). This program handles only homogeneous equilibria in solution and was considered adequate within the copper concentration and pH range of these bioassays. The copper species selected as being most important were Cu^{2+}, $CuOH^+$, $Cu(OH)_2^0$, $Cu_2(OH)_2^{2+}$, $CuHCO_3^+$, $CuCO_3^0$, and $Cu(CO_3)_2^{2-}$. The chemical equilibria considered in these calculations are presented in Table IV. The ionic strength calculated for the most concentrated test

Table II. Water Chemistry Conditions for Acute Copper Bioassays on Cutthroat Trout

	high alkalinity			medium alkalinity			low alkalinity		
hardness/alkalinity [a]	H/H	M/H	L/H	H/M	M/M	L/M	H/L	M/L	L/L
test no.	391	378	453	412	370	379	424	413	425
total hardness, mg/L $CaCO_3$									
av	205	69.9	18.0	204	83	31.4	160	74.3	26.4
(range)	(202–208)	(66.0–75.0)	(15.0–25.4)	(165–224)	(80–88)	(30.0–34.0)	(128–190)	(50.4–96.2)	(23.0–30.0)
total alkalinity, mg/L $CaCO_3$									
av	178	174	183	77.9	70	78.3	26.0	22.7	20.1
(range)	(173–188)	(142–222)	(150–270)	(74.4–80.0)	(66–72)	(58.0–114)	(24.6–28.0)	(21.0–24.6)	(19.0–20.4)
pH									
av	7.73	8.54	8.07	7.61	7.40	8.32	7.53	7.57	7.64
(range)	(7.69–7.77)	(8.27–8.77)	(7.62–8.65)	(7.50–7.78)	(6.60–7.70)	(8.22–8.57)	(7.32–7.60)	(7.39–7.76)	(7.38–7.79)
total Ca, mg/L									
av	49.8	18.4	4.8	64.7	20.4	7.9	57.5	24.7	6.0
(range)	(48.1–50.5)	(17.0–20.1)	(4.1–5.6)	(51.7–72.6)	(19.6–22.5)	(7.2–8.9)	(45.1–67.0)	(17.2–32.5)	(5.6–6.5)
total Mg, mg/L									
av (calcd)	19.6	5.8	1.5	10.3	7.8	2.7	4.0	3.1	2.8

[a] H = high, M = medium, L = low. Other parameters (averages and ranges over all bioassays): dissolved oxygen, 7.7 (6.7–8.9) mg/L; temperature, 13.7 (12.3–15.7) °C; ammonia nitrogen, 0.05 (0.00–0.17) mg/L; nitrate nitrogen, 0.05 (0.00–0.20) mg/L; nitrite nitrogen, all values 0.00 mg/L.

Table III. Multiplication Factors for Conversion of Nonfiltered to 0.45-μm Filtered Copper Concentrations as Measured by the Cuprethol Method

hardness/alkalinity [a]	H/H	M/H	L/H	H/M	M/M	L/M	H/L	M/L	L/L
hardness, mg/L $CaCO_3$	195	70	25	192	83	31	154	74	27
alkalinity, mg/L $CaCO_3$	160	174	169	72	70	78	20	23	20
pH	7.0	8.5	8.5	7.0	7.4	8.3	6.8	7.6	7.0
unfiltered Cu concns, mg/L	0.34–1.28	0.11–0.40	0.01–0.08	0.12–0.23	0.04–0.28	0.02–0.16	0.04–0.16	0.03–0.11	0.01–0.03
factor	0.92	0.94	0.89	0.82	0.87	0.86	0.78	0.79	0.74

[a] H = high, M = medium, L = low.

From: Environmental Science & Technology, September 1979.
Volume 13, No. 9, p. 1148

CORRESPONDENCE

SIR: In your February issue, C. Chakoumakos, R. C. Russo, and R. V. Thurston present 96-h LC_{50} acute copper toxicity data for "Cutthroat Trout (*Salmo clarki*) under Different Conditions of Alkalinity, pH, and Hardness" under nine combinations of these parameters (*ES&T*, 13, 213–9 (1979)). Data of this type are of great interest because they are cited by the EPA in regulatory actions, standards and guidelines, and environmental risk classification of chemicals. However, there are enormous differences between the effects of copper and other essential trace metals observed in these tests and those on aquatic life in the real environment.

The reason for such differences may be found in the conditions of the experiment, which are not designed to replicate anything in nature nor to indicate the actual risk to the environment, but are designed to place chemicals into neat and tidy categories for regulatory administrative purposes. It must be noted that, under the test conditions, the copper compounds in the water are highly unstable and undergo reactions of hydrolysis, association and precipitation. Specifically, in these tests a strong solution of a copper salt is mixed continuously with the water entering each experimental tank. The mean holding time in each tank was only 5 h, during which time from 8 to 22% of the copper was precipitated, according to Table III. This table indicates that the percentage of copper precipitated correlates poorly with alkalinity, hardness, or pH. This seems to betray evidence of inadequate experimental control.

Even overlooking this uncertainty, the practical application of such data appears to be limited to a situation such as that in a stream receiving a discharge of soluble copper. The toxicity data would then apply within a stream length representing the flow of water in 5 h. At points more remote from the point source, there would be a further reduction of copper concentration and a further change of speciation of the copper accompanied by a reduction of toxicity.

Because of the instability of the copper species under the experimental conditions of this study, the entire analysis of metal speciation in this publication is suspect, as is the attribution of degrees of toxicity to these species.

There are published studies by competent investigators of toxic effects of metals under natural environmental conditions on numerous aquatic species. One wonders what purpose is served by highly contrived test conditions that appear to be favored by regulatory agencies. These place an undue onus on minor discharges or spills that are, in fact, of little actual consequence to the environment.

In conclusion, I would like to suggest that a possible reason for the inability to control the rate of precipitation of copper in these experiments may be the presence of uncontrolled small amounts of suspended matter in the water. Suspended particles behave as nuclei for precipitation. Suspended matter is always present in natural waters and it greatly reduces the toxic risk of copper to aquatic life.

S. B. Tuwiner

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SIR: Dr. Tuwiner appears to raise two points about the subject paper. The first relates to a time factor required to reach equilibrium and the transitory nature of that equilibrium in natural environments. Equilibrium for the copper system studied was effectively attained far short of the tank water residence time. We based our calculations on the total soluble copper under the conditions specified.

His second point relates to oversimplifications of the complexities of natural aquatic systems that result from placing chemicals "into neat and tidy categories for regulatory administrative purposes". We believe Tuwiner is expressing recognition of a point already illustrated in the subject paper.

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Table IV. Equilibria for Copper Test Water Solutions (*28*)

reaction	log K_{eq} [a]
$H^+ + OH^- \rightleftarrows H_2O$	14.0
$H^+ + CO_3^{2-} \rightleftarrows HCO_3^-$ (K_2)	10.33
$2H^+ + CO_3^{2-} \rightleftarrows H_2CO_3$ ($K_1 + K_2$)	16.68
$Ca^{2+} + H_2O \rightleftarrows CaOH^+ + H^+$	−12.7
$Ca^{2+} + H^+ + CO_3^{2-} \rightleftarrows CaHCO_3^+$ (1.0 + K_2)	11.33
$Ca^{2+} + CO_3^{2-} \rightleftarrows CaCO_3^0$	3.15
$Mg^{2+} + H_2O \rightleftarrows MgOH^+ + H^+$	−11.42
$Mg^{2+} + H^+ + CO_3^{2-} \rightleftarrows MgHCO_3^+$ (0.95 + K_2)	11.28
$Mg^{2+} + CO_3^{2-} \rightleftarrows MgCO_3^0$	2.88
$Cu^{2+} + H_2O \rightleftarrows CuOH^+ + H^+$	−7.7
$Cu^{2+} + 2H_2O \rightleftarrows Cu(OH)_2^0 + 2H^+$	−14.32 [b]
$2Cu^{2+} + 2H_2O \rightleftarrows Cu_2(OH)_2^{2+} + 2H^+$	−10.3
$Cu^{2+} + H^+ + CO_3^{2-} \rightleftarrows CuHCO_3^+$	12.3 [c]
$Cu^{2+} + CO_3^{2-} \rightleftarrows CuCO_3^0$	6.75
$Cu^{2+} + 2CO_3^{2-} \rightleftarrows Cu(CO_3)_2^{2-}$	9.92

[a] Constants determined at 18–25 °C and corrected to zero ionic strength. [b] Constant from Vuceta and Morgan (*29*). [c] Constant from Childs (*30*).

water was 5.38×10^{-3} M. The equilibrium constant, log K_2, for the bicarbonate–carbonate system calculated with this correction is 10.20, as compared to the uncorrected value of 10.33. Considering the variability of equilibrium constants available in the literature, this difference was considered negligible and the equilibrium constants for the reactions presented in Table IV were used without correction. The other variables introduced into COMICS included pH and the molar concentrations of total inorganic carbon, calcium, and magnesium. Total inorganic carbon (moles/liter) was calculated from bicarbonate alkalinity and pH using the following equations:

$$[\text{inorganic C}] = \frac{[HCO_3^-]([H^+] + K_1)}{K_1}$$

$$[\text{inorganic C}] = \frac{[HCO_3^-]([H^+] + K_2)}{[H^+]}$$

Results and Discussion

The 96-h LC_{50} values and their 95% confidence interval (C.I.) end points for the bioassays are presented in Table V, and the toxicity curves for these bioassays are shown in Figure 1; LC_{50} values for time periods other than 96 h can be estimated from these curves. The curves illustrate that the 96-h test period was sufficient to attain an asymptotic LC_{50}, also known as the incipient LC_{50} (*31*).

Fish size data are also presented in Table V. Although the test fish were all obtained from the same pool, the tests were conducted over a 3-month period during which fish grew in size prior to testing. We have no data relating the susceptibility of cutthroat trout of different sizes to copper toxicity under uniform water chemistry conditions, but in 11 separate bioassays which were under uniform water chemistry conditions we have studied this relationship using 1- to 26-g rainbow trout as the test fish. These data are presented in Table VI. There was a small but significant effect on the susceptibility of the rainbow trout to copper toxicity based on fish size; the larger fish were slightly less susceptible than the smaller fish. The correlation coefficient for the relationship between fish weight and the observed LC_{50} values was 0.68 ($P = 0.009$), and the equation of the regression line was $LC_{50} = (0.1266)\cdot(1.0374)^{\text{weight}}$. Howarth and Sprague (*17*) have recently reported that 10-g rainbow trout were 2.5 times more resistant to copper than 0.7-g trout. Considering the narrower range of fish weight (3–10 g) in our cutthroat trout bioassays (Table V), it was assumed that fish size in our experiments was not significantly related to susceptibility to copper toxicity.

Table V. Acute Copper Toxicity to Cutthroat Trout under Different Conditions of Alkalinity and Hardness (10 Fish/Tank)

test no.	fish size: wt, g	fish size: length, cm	alkalinity	hardness	96-h LC_{50} (95% C.I.), mg/L Cu_{total} [a]
391	4.2	7.4	high	high	0.367 (0.319–0.424)
378	3.2	6.9	high	medium	0.186 (0.172–0.202)
453	9.7	8.8	high	low	0.0368 (0.0350–0.0387)
412	4.4	8.1	medium	high	0.232
370	2.7	6.8	medium	medium	0.162 (0.148–0.176)
379	3.2	7.0	medium	low	0.0736 (0.0672–0.0805)
424	5.2	8.5	low	high	0.0910 (0.0832–0.0996)
413	4.4	7.7	low	medium	0.0444 (0.0403–0.0489)
425	5.7	8.9	low	low	0.0157

[a] Cu_{total} as measured by cuprethol on 0.45-μm filtrate.

Table VI. Acute Copper Toxicity to Rainbow Trout of Different Sizes, under Uniform Water Chemistry Conditions [a]

test no.	no. fish per tank	fish size: wt, g	fish size: length, cm	96-h LC_{50} (95% C.I.), mg/L Cu_{total} [b]
128	20	1		0.169 (0.148–0.193)
496	20		4.9	0.0853 (0.0767–0.0950)
403	10	2.1	6.0	0.0833 (0.0748–0.0928)
503	20	2.5	6.1	0.103 (0.0961–0.111)
169	20	2.6		0.274 (0.222–0.337)
136	20	4.3		0.128 (0.101–0.162)
141	20	9.4	9.2	0.221 (0.201–0.243)
236	5	11.5	9.9	0.165 (0.137–0.198)
233	5	18.7	11.8	0.197 (0.152–0.254)
166	10	24.9	13.5	0.514 (0.434–0.610)
160	10	25.6	13.4	0.243 (0.210–0.281)

[a] Water chemistry data averaged over all tests (averages with ranges in parentheses): pH 7.84 (7.77–7.91); alkalinity, 174 (173–176) mg/L $CaCO_3$; hardness, 194 (192–196) mg/L $CaCO_3$; calcium, 55.1 (49.7–61.4) mg/L; magnesium (calculated), 13.7 mg/L; dissolved oxygen, 7.9 (5.6–9.6) mg/L; temperature, 12.8 (11.8–13.9) °C; ammonia nitrogen, 0.07 (0.00–0.27) mg/L; nitrate nitrogen, 0.12 (0.00–0.31) mg/L; nitrite nitrogen, all 0.00 mg/L. [b] Cu_{total} as measured by cuprethol on 0.45-μm filtrate.

The copper LC_{50} values presented in Table V verify the long-standing observation that the toxicity of copper to fishes is inversely correlated with the hardness of water. Results of the COMICS equilibrium calculations using the copper 96-h LC_{50} values (in molarity) are presented in Table VII, and percent distributions of the copper species are given in Table VIII. Hardness and alkalinity determined the magnitude of the copper LC_{50} value. The estimated partial correlation between the copper LC_{50} value and hardness, holding alkalinity fixed, is 0.882 (90% C.I. = 0.571–0.972); the estimated partial correlation between the copper LC_{50} value and alkalinity, holding hardness fixed, is 0.756 (90% C.I. = 0.246–0.938). The effect of alkalinity on the LC_{50} may be due in part to its effect on copper speciation, but cannot be explained entirely on this basis. Alkalinity and pH independently control the speciation of copper.

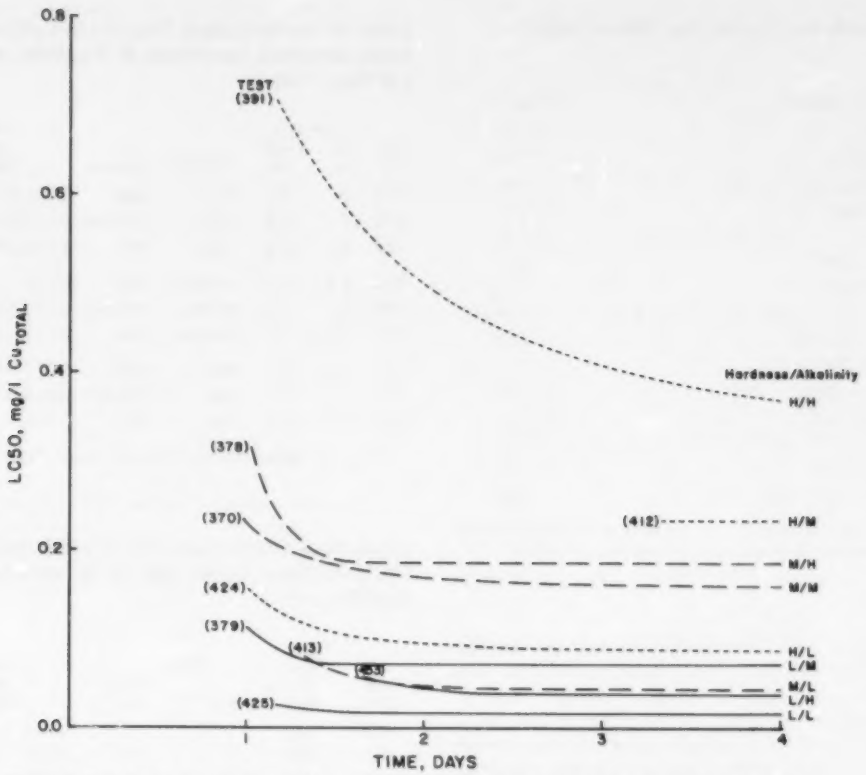

Figure 1. Toxicity curves for copper bioassays on cutthroat trout (H = high, M = medium, L = low)

Table VII. Metal Speciation for Copper 96-h LC_{50} Values for Cutthroat Trout (Concentrations in Moles/Liter)

	hardness/alkalinity [a] and test no.								
	H/H 391	M/H 378	L/H 453	H/M 412	M/M 370	L/M 379	H/L 424	M/L 413	L/L 425
Cu_T [b] ($\times 10^8$)	578	293	57.9	365	255	116	143	69.9	24.7
Ca_T [c] ($\times 10^4$)	12.4	4.59	1.20	16.1	5.09	1.97	14.3	6.16	1.50
Mg_T [c] ($\times 10^4$)	8.07	2.39	0.617	4.24	3.21	1.11	1.65	1.28	1.15
HCO_3^- [d] ($\times 10^4$)	35.6	34.8	36.6	15.6	14.0	15.7	5.20	4.54	4.02
inorganic C ($\times 10^4$)	37.1	35.4	37.3	16.4	15.2	15.9	5.54	4.81	4.23
pH	7.73	8.54	8.07	7.61	7.40	8.32	7.53	7.57	7.64
CO_3^{2-} ($\times 10^8$)	871	5510	2000	289	162	1510	80.7	78.0	81.7
Ca^{2+} ($\times 10^8$)	118 000	41 300	11 300	158 000	50 100	19 000	142 000	61 300	14 900
$CaOH^+$ ($\times 10^8$)	1.27	2.86	0.264	1.28	0.251	0.792	0.961	0.454	0.130
$CaHCO_3^+$ ($\times 10^8$)	4110	1400	409	2390	692	294	724	275	59.7
$CaCO_3^0$ ($\times 10^8$)	1460	3210	318	644	115	406	162	67.5	17.2
Mg^{2+} ($\times 10^8$)	77 800	22 300	5890	41 700	31 700	10 800	16 400	12 700	11 500
$MgOH^+$ ($\times 10^8$)	15.9	29.4	2.63	6.46	3.02	8.59	2.11	1.80	1.90
$MgHCO_3^+$ ($\times 10^8$)	2400	675	191	563	390	149	74.5	51.0	40.9
$MgCO_3^0$ ($\times 10^8$)	514	931	89.1	91.4	39.0	124	10.0	7.54	7.10
Cu^{2+} ($\times 10^8$)	8.78	0.319	0.313	13.9	18.5	0.385	12.1	5.47	1.58
$CuOH^+$ ($\times 10^8$)	9.41	2.21	0.733	11.3	9.26	1.61	8.21	4.05	1.38
$Cu(OH)_2^0$ ($\times 10^8$)	121	183	20.6	111	55.8	80.5	66.7	36.1	14.4
$Cu_2(OH)_2^{2+}$ ($\times 10^8$)	0.111	0.00612	0.000676	0.161	0.108	0.00324	0.0848	0.0207	0.00238
$CuHCO_3^+$ ($\times 10^8$)	2.84	0.101	0.106	1.97	2.38	0.0556	0.577	0.229	0.0590
$CuCO_3^0$ ($\times 10^8$)	430	98.8	35.1	226	169	32.8	55.1	24.0	7.26
$Cu(CO_3)_2^{2-}$ ($\times 10^8$)	5.54	8.06	1.03	0.964	0.404	0.733	0.0658	0.0277	0.00878

[a] H = high, M = medium, L = low. [b] $Cu_T = Cu_{total}$ as measured by cuprethol on 0.45-μm filtrate. [c] $Ca_T + Mg_T$ = hardness. [d] HCO_3^- = alkalinity.

The most abundant forms of soluble copper over all the conditions tested were dissolved copper(II) carbonate, $CuCO_3^0$, and copper(II) hydroxide, $Cu(OH)_2^0$. The amount of copper(II) ion dramatically increased as the alkalinity and pH decreased, and a more subtle, but parallel, behavior was exhibited by $CuOH^+$ within the pH range tested. In contrast

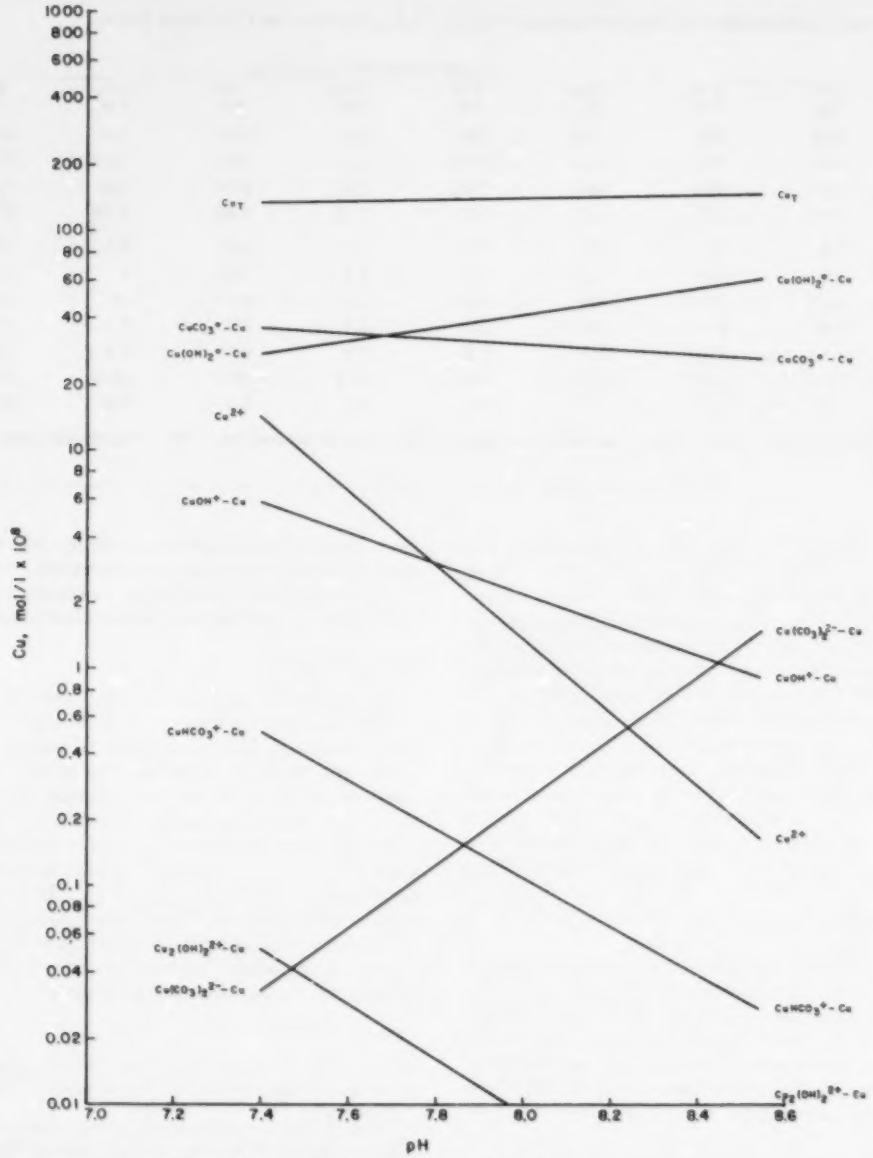

Figure 2. 96-h LC_{50} copper species distribution diagram for bioassays on cutthroat trout
Regression equations: log $[Cu^{2+}]$ = 13.6762 − 1.692pH (P = 0.001); log $[CuOH^{+}\text{-}Cu]$ = 5.8700 − 0.691pH (P = 0.074); log $[Cu(OH)_2^0\text{-}Cu]$ = −0.8204 + 0.307pH (P = 0.384); log $[Cu_2(OH)_2^{2+}\text{-}Cu]$ = 8.9548 − 1.385pH (P = 0.074); log $[CuHCO_3^{+}\text{-}Cu]$ = 7.9767 − 1.117pH (P = 0.072); log $[CuCO_3^0\text{-}Cu]$ = 2.4379–0.118pH (P = 0.830); log $[Cu(CO_3)_2^{2-}\text{-}Cu]$ = −12.2673 + 1.457pH (P = 0.11); log $[Cu_T]$ = 1.8412 + 0.040pH (P = 0.926)

to these four species, the amounts of $Cu_2(OH)_2^{2+}$, $CuHCO_3^{+}$, and $Cu(CO_3)_2^{2-}$ were very small. Within the pH range tested, the concentration of $Cu_2(OH)_2^{2+}$ increased with a decrease in alkalinity. The concentration of $CuHCO_3^{+}$ increased with a decrease in pH. $Cu(CO_3)_2^{2-}$ was more abundant at high pH and high alkalinity.

Interpretation of the results of the experimental tests reported in this paper, along the above lines of reasoning which lead to prioritizing chemical species in order of relative toxicity, is constrained by the variation of pH among the tests conducted. However, pH was relatively constant in the three bioassays conducted at high hardness coupled with a high, medium, and low alkalinity (Table VIII: tests 391, 412, and 424). These data show that as the proportions of carbonate species decreased, the median lethal concentration of copper also decreased; as the proportions of Cu^{2+} and the hydroxyl species increased, the LC_{50} decreased. This behavior eliminates $CuCO_3^0$ as a toxic form of copper, supporting the hypothesis of Stiff (*11*) and the findings of Howarth and Sprague (*17*), but in disagreement with the conclusions of Shaw and Brown (*9*); it also eliminates $CuHCO_3^{+}$ and $Cu(CO_3)_2^{2-}$. It corroborates the widely held view that Cu^{2+} is toxic and also implicates $CuOH^{+}$ and $Cu(OH)_2^0$ as toxic forms. The data indicate that $Cu_2(OH)_2^{2+}$ may also be toxic, as has recently been reported by Andrew et al. (*16*) and Howarth and Sprague (*17*); however, the concentration of $Cu_2(OH)_2^{2+}$ is two to four orders of magnitude smaller than Cu^{2+}, $CuOH^{+}$, and $Cu(OH)_2^0$, so its acute toxic effect may be negligible by comparison. The evidence from this study that $CuOH^{+}$ is toxic to fishes is in agreement with the conclusions of Pagenkopf et al. (*15*), Andrew et al. (*16*), and Howarth and Sprague (*17*). The present work further suggests that $Cu(OH)_2^0$ may also be toxic to fishes.

Examination of the data in Tables VII and VIII shows that

Table VIII. Percent Distribution of Copper Species for 96-h LC_{50} Values for Cutthroat Trout

	hardness/alkalinity [a] and test no.								
	H/H 391	M/H 378	L/H 453	H/M 412	M/M 370	L/M 379	H/L 424	M/L 413	L/L 425
Cu_T [b]	578	293	57.9	365	255	116	143	69.9	24.7
alkalinity [c]	178	174	183	77.9	70.0	78.3	26.0	22.7	20.1
hardness [c]	205	69.9	18.0	204	83.0	31.4	160	74.3	26.4
pH	7.73	8.54	8.07	7.61	7.40	8.32	7.53	7.57	7.64
Cu^{2+}	1.5	0.1	0.5	3.8	7.2	0.3	8.5	7.8	6.4
$CuOH^+$	1.6	0.8	1.3	3.1	3.6	1.4	5.7	5.8	5.6
$Cu(OH)_2^0$	20.9	62.4	35.6	30.4	21.9	69.4	46.6	51.6	58.3
$Cu_2(OH)_2^{2+}$	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
$CuHCO_3^+$	0.5	0.0	0.2	0.5	0.9	0.0	0.4	0.3	0.2
$CuCO_3^0$	74.4	33.7	60.6	61.9	66.3	28.3	38.5	34.3	29.4
$Cu(CO_3)_2^{2-}$	1.0	2.8	1.8	0.3	0.2	0.6	0.0	0.0	0.0

[a] H = high, M = medium, L = low. [b] $Cu_T = Cu_{total}$ as measured by cuprethol on 0.45-μm filtrate; in moles/liter × 10^6. [c] Alkalinity and hardness in milligrams/liter as $CaCO_3$.

(1) at constant alkalinity it is the pH that determines the relative distribution of Cu^{2+}, $CuOH^+$, and $Cu(OH)_2^0$; (2) at a constant pH, it is the alkalinity that determines the relative distribution of Cu^{2+}, $CuOH^+$, and $Cu(OH)_2^0$; (3) the highest concentrations of Cu^{2+} and $CuOH^+$ occurred at the low pHs and low alkalinities; (4) the concentration of Cu^{2+} is sensitive to changes in pH; (5) the percents of Cu^{2+} and $CuOH^+$ are the same at pH 7.7; above pH 7.7 $CuOH^+$ is the predominant of those two species, whereas below pH 7.7 Cu^{2+} predominates; and (6) the proportion of $Cu(OH)_2^0$ increases with increasing pH. To illustrate these observations in a more generalized manner, the data were used to construct the copper species distribution diagram shown in Figure 2. In this figure, the LC_{50} concentrations for each test of the six copper species $CuOH^+$, $Cu(OH)_2^0$, $Cu_2(OH)_2^{2+}$, $CuHCO_3^+$, $CuCO_3^0$, and $Cu(CO_3)_2^{2-}$ are expressed as their concentrations of Cu; the sum total of these is the LC_{50} as total dissolved copper.

In summary, hardness and alkalinity control the median lethal concentration of copper toxic to cutthroat trout. The most important copper species causing toxicity within the pH range tested are Cu^{2+}, $CuOH^+$, and $Cu(OH)_2^0$. The concentration of each of these species varies with pH and alkalinity. Lower pHs favor Cu^{2+}; higher pHs favor $CuOH^+$ and $Cu(OH)_2^0$. Lower alkalinities favor all three of these species.

It is concluded that the critical factors regarding copper toxicity to fishes are not so much which copper species are toxic, as are the controlling roles of hardness, alkalinity, and pH. To ensure the protection of aquatic life, it is recommended that consideration be given to establishing water quality criteria for copper not only on the measure of total copper, but also based on a stepwise scale dictated by the pH, hardness, and alkalinity of a given aquatic environment.

Note Added in Proof

The constants used in these calculations were the best available in the literature. Recent unpublished data (W. G. Sunda, personal communication ,1978) raises some question concerning the formation constant for $Cu(OH)_2^0$. The calculations upon which the toxicity conclusions reported above are based have been redone using the alternate value for the formation constant for $Cu(OH)_2^0$. The effect of this is to reduce $Cu(OH)_2^0$ from a major to a minor species in the distribution of constituents, but the relative concentrations of all species, $Cu(OH)_2^0$ included, as a function of alkalinity, hardness, and pH, remain unchanged. Since the toxicity conclusions are based entirely upon trends in the concentrations of the various chemical species, these conclusions are valid regardless of which constant may ultimately prove to be correct. We wish to thank K. Emerson for assistance with recalculation of the data for comparison with our initial findings.

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